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Full Length Research Article

SERO-PREVALENCE OF HEPATITIS B MARKERS AMONG BLOOD DONORS FROM NATIONAL BLOOD TRANSFUSION SERVICE CENTRE IN KADUNA, NIGERIA

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ABSTRACT
The survey was carried out to determine the prevalence of Hepatitis B virus markers among blood donors from National Blood Transfusion Service (NBTS) centre in Kaduna, Nigeria. Five milliliters of blood was collected through the vein into the EDTA container and screened for Hepatitis B virus (HBV) using diagnostic kit for HBV infection marker in whole blood. The socio-demographic factors associated with HBV infection were determined using structured questionnaires. The prevalence of HBV markers among blood donors was compared using Pearson correlation matrix. The total prevalence rates of the five (5) HBV markers were: HBsAg 12(7.3 %), HBsAb 5(3.0 %), HBeAb 12(7.3 %), HBcAb 18(10.9 %) while all blood samples were negative for HBeAg. Statistically, there were significant differences between the rate of prevalence for HBsAb and age, likewise HBcAb and occupation at ($p<0.05$). A high significant correlation was found between HBsAg and HBcAb at $r=0.725$ and $p=0.0001$, between HBsAg and HBeAb at $r=0.820$ and $p=0.0001$ as well. Additionally, there was a high significant correlation between HBcAb and HBsAb at $r=0.278$ and $p=0.0001$, between HBcAb and HBeAb at $r=0.725$ and $p=0.0001$. However, HBsAg and HBcAb have no significant correlation with any other HBV markers ($P>0.01$). Some of the samples that were HBsAg negative have been found to be positive for other HBV markers specifically HBcAb (IgG and IgM). Therefore, based on this study screening for HBsAg alone might not be sufficient for diagnosis of HBV infection.

Keywords: Sero-prevalence, Hepatitis B virus (HBV), Blood donors, National Blood Transfusion Service (NBTS)

INTRODUCTION

One of the most essential and crucial section of the medical services is blood transfusion. The prime concern of the blood transfusion service is to ensure the preservation, intelligibility competence, and efficacy of blood supplies at all stages (Islam, 2009). The freight of hepatitis B infection in major part of the developing countries particularly in rural areas is hefty; this freight is constituted by the lofty cost of management, treatment and prevention (Rosa *et al.,* 2015). Hepatitis B virus (HBV) is a double stranded circular DNA virus, belongs to the family Hepadnaviridae (Prescott *et al.,* 2008). It possesses a complicated structure and is amongst the most contagious diseases worldwide (Blattacharya *et al.,* 2007), emerging in fatal chronic liver diseases. According to World Health Organization (WHO), the present universal burden of hepatitis B infection have been predicted about two billion, from whom three hundred and sixty million have chronic infection by end of 2016, whereas six hundred and twenty thousand dies yearly (Wiktor and Hutin, 2016). Recently, WHO estimated that two hundred and fifty-seven million individuals are carriers of hepatitis B infection that is, $HBsAg^+$ (WHO, 2019). Similarly, HBV is responsible for higher number of deaths as compared to malaria, tuberculosis and HIV yearly (Wiktor and Hutin, 2016). HBV infection is among the most frequent cause of liver disease and is a serious health challenge in Nigeria (Musa *et al.,* 2015). Seventy-five (75) percent of the total population is discovered to be infected with hepatitis B virus at some point in their life, with approximately 12 % prevalence of chronic carriers (Alao *et al.,* 2009; Oladele *et al.,* 2013). The incubation period of hepatitis B virus is 30-180 days (Chang, 2007). HBV continues to be the leading threat for secure blood usage (Ikerionwu, 2018). Individuals that are not immunized can easily pass on the hepatitis B virus, through contact with contaminated blood or body fluids like semen, vaginal discharge, saliva, menstrual fluid and blood (Rubin and Strayer, 2015). Hence they are acquired through contaminated blood transfusion, intercourse specifically sex without condoms, exchanging sharp infected items like syringes, scalpel, as well as personal contact in congested environment (Rubin and Strayer, 2015). It can also be passed on from an infectious mother to the fetus via the placental barrier. Many people at the time of initial diagnosis are asymptomatic, but few result in a rapid progression of the disease along with dark urine, throwing up, fatigue, stomach pain and yellowish skin. These symptoms will usually continue for a few days, and the primary disease rarely progresses to death (Rubin and Strayer, 2015). HBcAb IgG is helpful for detecting chronic HBV infection, while HBcAb IgM is helpful in detecting recent HBV infection. Hepatitis B surface antigen has the ability to produce viral particles which have a complicated surface antigen and is extensively disseminated in the bloodstream of infected individuals. There is a period when hepatitis B surface antigen can't be detected within the bloodstream, even though HBV infection still exists in the bloodstream. During the window phase, recognized antibody produced in response to hepatitis B core antigen functions like an effective serological marker for HBV infection (Bharath and Krishnan, 2016). Lack of Hepatitis B surface antigen in bloodstream of possibly active people might not be adequate to assure lack of disseminating HBV, so blood consisting of anti-HBc either with or without observable appearance of HBsAg could be infectious (Japhet, 2011; Jeremiah, 2011). HBV residual volume is higher through transfusion, this is ascribed to the period between initial HBV infection and observation of HBsAg, which result into a long window period at the same time in which the virus is transmitted (Durro and Onyra, 2011; Jeremiah *et al.,* 2011). HBV infection

may either be acute or chronic hepatitis (Gerlich, 2013). This study is concerned primarily with the sero-prevalence of hepatitis B virus among blood donors from National Blood Transfusion Service centre in Kaduna, Nigeria.

MATERIALS AND METHODS

Subjects

The study included apparently healthy blood donors both males and females who came to donate blood in national blood transfusion service (NBTS) centre in Kaduna, Nigeria between July to September 2019.

The sample size was determined using Cochran's formula

$$n = \frac{t^2 \times p(1-p)}{m^2}$$

(Cochran, 1977).

Where n is the required sample size, t represented confidence level at 95% (standard value of 1.96), p represented 12% (Edia-Asuke *et al.,* 2016), average prevalence of HBsAg+ in Nigeria from previous studies, m equaled 5% (standard value of 0.05) margin of error. A total of 165 donor samples were taken. Therefore, 165 blood samples were collected from prospective blood donors. All the Institutional and ministry of health ethical clearance for this study was given by the department of Microbiology, Kaduna State University of Science and Ministry of Health Kaduna. All the participants gave their written informed consents before blood samples were collected from them.

Sample Collection

Five milliliters of blood were collected through the vein into the EDTA container. The cassette was kept to the right side matching to HBsAg, HBsAb, HBeAg, HBeAb HBcAb. With a small pipette the whole blood sample was taken and added into the five (5) wells of the cassette by dropping (25µl per well or one drop), Then one drop of the buffer was added into the five (5) sample well as well. The interpretation of test results was performed according to manufacturer's description. The plasma for positive blood samples was separated through centrifugation at 5000rpm for 15 minutes

Detection of Hepatitis B Markers

For hepatitis B virus, a diagnostic kit for HBV infection marker in whole blood manufactured by micro point technologies Inc U.S.A was used in a stepwise order for the detection of HBsAg, HBsAb, HBeAg, HBeAb and HBcAb respectively in the blood. This method which is immunochromatographic and qualitative in nature, detects the presence of five markers of hepatitis B virus in human blood and can be read in-vitro having more than 99.9 % sensitivity and 99.75 % specificity.

Socio-demographic Profile

A structured questionnaire was used to obtain information on socio-demographic factors (gender, age and occupation).

Statistical Analysis

The data was subjected to statistical analysis and chi square test was carried out using SPSS computer software version 23.0 for windows to determine any significant relationship between age, gender and occupation for the different markers of hepatitis B virus.

RESULTS

The Prevalence of Hepatitis B Markers among Blood Donors

One hundred and sixty-five blood donors took part in this study at the NBTS centre Kaduna. Of the 165 blood samples tested, HBsAg was detected in 12(7.3 %), HBsAb 5(3.0 %), HBeAb 12(7.3 %), and HBcAb 18(10.9 %). All blood samples were negative for HBeAg. The total prevalence of positive and negative markers detected were 7.13 % and 94.3 % respectively (Table 1).

Table 1: The Prevalence of Hepatitis B Markers among Blood Donors.

HBV Markers	Total samples	Total positive %	Total negative %
HBsAg	165	12(7.3)	153(92.7)
HBsAb	165	5(3.0)	160(97.0)
HBeAb	165	12(7.3)	153(92.7)
HBcAb	165	18(10.9)	147(89.1)

Twenty-eight female blood donor's participated in this study, of which 3(1.8 %), 1(0.6 %), 2(1.2 %), 3(1.8 %) were positive for HBsAg, HBsAb, HBeAb and HBcAb respectively. Of the one hundred and thirty-seven males who participated in this study, 9(5.5 %), 4(2.4 %), 10(6.1 %), 15(9.1 %) were positive for HBsAg, HBsAb, HBeAb and HBcAb respectively. Both females and male donors have been negative for HBeAg. This result showed no significant difference ($p<0.05$) among the distribution of hepatitis B markers between the blood donors based on gender (Table 2).

Socio-demographic Profile

The prevalence of Hepatitis B Markers among blood donors in relation to gender, age and occupation are presented as follows;

Table 2: The Prevalence of Hepatitis B Markers among Blood Donors by Gender

Gender		Hepatitis B Virus (HBV) Positive Markers			
	N	HBsAg n(%)	HBsAb n(%)	HBeAb n(%)	HBcAb n(%)
Female	28	3(1.8)	1(0.6)	2(1.2)	3(1.8)
Male	137	9(5.5)	4(2.4)	10(6.1)	15(9.1)
χ^2		0.592	0.034	0.001	0.001
P-value		0.442	0.855	0.977	0.971
OR		1.707	1.231	0.977	0.976
CI		0.431-6.751	0.132-11.453	0.202-4.723	0.263-3.625

In terms of age, hepatitis B markers were significantly higher in age group 18-27 years as related with other age group. Also, those within 18-27 years have high positivity of 4(2.4%),5(3.0 %), 5(3.0 %) for HBsAg, HBeAb and HBcAb respectively. Additionally, those with age group 58+ had lower positivity for HBV markers as compared with other age groups. All age groups have shown no significant differences ($p<0.05$) in relation to HBsAg, HBeAb and HBcAb whereas all age groups showed highly significant association with HBsAb (χ^2= 13.373, df= 4, p= 0.010). Furthermore, all age groups were negative for HBeAg (Table 3).

Table 3: Prevalence of Hepatitis B Markers among Blood Donors by Age

Age(years)	Hepatitis B Virus (HBV)Positive Markers				
	N	HBsAg	HBsAb	HBeAb	HBcAb
		n(%)	n(%)	n(%)	n(%)
18-27	31	4(2.4)	0(0.0)	5(3.0)	5(3.0)
28-37	48	2(1.2)	1(0.6)	3(1.8)	3(1.8)
38-47	50	3(1.8)	0(0.0)	2(1.2)	3(1.8)
48-57	25	2(1.2)	2(1.2)	2(1.2)	4(2.4)
58+	11	1(0.6)	2(1.2)	0(0.0)	3(1.8)
χ^2		2.338	13.373	5.356	6.878
P-value		0.674	0.010	0.253	0.142

In regard to occupation, blood donors who are self-employed have significantly higher HBV markers as compared with other line of works. Students and blood donors with other occupations have similar positivity of 3(1.8 %) for HBsAg while civil servants have high positivity of 2(1.2 %) for HBsAb. Students have high positivity of 4(2.4 %) for HBeAb whereas self-employed have high positivity of 5(3.0 %) for HBcAb. All line of works has shown no significant difference ($p<0.05$) in relation to HBsAg, HBsAb and HBeAb. Although all line of works showed highly significant association with HBcAb ($\chi^2=10.860$, df= 4, $p=0.028$). On the other hand, all line of works were negative for HBeAg (Table 4).

Table 4: Prevalence of Hepatitis B Markers among Blood Donors Based on Occupation

Occupation	Hepatitis B Virus (HBV)Positive Marker				
	N	HBsAg	HBsAb	HBeAb	HBcAb
		n(%)	n(%)	n(%)	n(%)
	37	3(1.8)	0(0.0)	4(2.4)	3(1.8)
Student					
civil servant	45	2(1.2)	2(1.2)	1(0.6)	3(1.8)
Self Employed	56	2(1.2)	1(0.6)	3(1.8)	5(3.0)
Unemployed	10	2(1.2)	1(0.6)	2(1.2)	4(2.4)
Others	17	3(1.8)	1(0.6)	2(1.2)	3(1.8)
χ^2		6.825	3.881	5.604	10.860
P-value		0.145	0.422	0.231	0.028

There was high significant correlation amongst HBsAg and HBcAb at r=0.725 and p=0.0001. Also between HBsAg and HBeAb at r=0.820 and p=0.0001. Additionally, there was high significant correlation between HBcAb and HBsAb at r=0.278 and p=0.0001, as well as between HBcAb and HBeAb at r=0.725 and p=0.0001 while HBsAg and HBcAb have no significant correlation with any other HBV markers (Table 5).

Table 5: Pearson Correlation Matrix of the Hepatitis B Virus (HBV) Markers

		HBsAg	HBcAb	HBsAb	HBeAb
HBsAg	R	1	.725**	-.050	.820**
	p-value		.000	.528	.000
HBcAb	R	.725**	1	.278**	.725**
	p-value	.000		.000	.000
HBsAb	R	-.050	.278**	1	.087
	p-value	.528	.000		.269
HBeAb	R	.820**	.725**	.087	1
	p-value	.000	.000	.269	

**Correlation is significant at the p<0.01

DISCUSSION

In this study, 7.3 % prevalence of HBsAg detected in 165 blood samples analyzed is an indication of the occurrence of HBV among blood donors from NBTS centre in Kaduna, Nigeria. This moreover indicates that HBV infection is endemic in the region of the study, and this might be ascribed to not receiving the complete doses of the vaccine and also due to individual behavior and practices in the studied area. The prevalence of HBsAg reported in this study is lower than 4.87 % as stated in a previous study which was 12 % as reported by Edia-Asuke *et al.* (2016).

Also the prevalence of 7.3 % stated in this study is lower than 0.4 % as reported in a previous study which was 7.7 % as carried out by Ikerionwu (2018).

According to gender differences, it has shown that female blood donors had higher seropositivity for the HBV markers than the male counterpart, although the difference was not statistically significant ($p>0.05$). The number of males higher than female is due to the fact that male donate more blood than the females, issue like menstrual cycle and child birth are some of the reasons why females donate lesser that males. The cause for this difference may be because of the high number of male blood donors enrolled for this study than females. The result in this study correspond to Edia-Asuke *et al.* (2016), who stated that females are more infected with HBsAg than the male counterpart. Other reason for the high infection rate among females might be due to multiple sexual partnership, having unprotected sex and other unhygienic activities engaged by the youth (Lawal, 2009; Augustine *et al*., 2014; Innocent *et al.,* 2015). This result showed no significant differences ($p<0.05$) in relation to the HBV markers which is similar to Innocent *et al.* (2015). However, this study contradicts the previous findings by Ndako *et al.* (2016), which reported higher prevalence for HBsAg in male than in females. This is quite high in view of the fact that the study subjects seem to be healthy population. Similar result was recorded in Lagos, Nigeria by Balogun (2010). A related survey also stated higher hepatitis B surface antigen prevalence in males than females among patients attending dental Clinic in University College Hospital, Ibadan, Nigeria, this might be due shorter carrier rate of HBsAg in females compared to males as reported by Lawal (2009).

The highest prevalence of Hepatitis B markers is detected among the blood of donors between the age group of 18-27 years old. This high prevalence rate in younger people may be related to their active sexual activities and drug abuse, similar to the result

reported by Buseri *et al.,* (2009). This study contradicts the earlier report with high prevalence of HBV in older subjects 40 years and above than in younger people Lawal (2009), Okonko (2011) and Innocent *et al.* (2015). All age groups showed highly significant association with HBsAb (χ^2 = 13.373, df= 4, p= 0.010). HBsAb presence can be due to the fact that it develops in individuals who have been vaccinated for Hepatitis b infection, so this can be the reason why these individuals are positive for HBsAb.

In regard to occupation, blood donors who are self-employed have significantly higher positivity for HBV markers as compared with other line of works. This study contradicts what had been previously reported by Augustine *et al.* (2014), which stated that students have low prevalence of HBsAg whereas in this study students have high positivity for HBsAg. All line of work showed highly significant association with HBcAb (χ^2 = 10.860, df= 4, p= 0.028). HBcAb indicates a previous or ongoing infection and this might be due to exposure, the kind of work people get involve into can lead to hepatitis B infection.

There was high significant correlation amongst HBsAg and HBcAb at r=0.725 and p=0.0001. Also between HBsAg and HBeAb at r=0.820 and p=0.0001. Additionally, there was high significant correlation between HBcAb and HBsAb at r=0.278 and p=0.0001, as well as between HBcAb and HBeAb at r=0.725 and p=0.0001 while HBsAg and HBcAb have no significant correlation with any other Hepatitis B virus (HBV) markers. This work contradicts the work of Innocent *et al.* (2015), who reported a strong correlation between HBsAg and HBeAg.

Conclusion

The study revealed an overall low prevalence of Hepatitis B markers among healthy blood donors from National Blood Transfusion Service Centre in Kaduna. Female blood donors had higher seropositivity for the HBV markers than the male counterpart but the differences were not significant. The highest prevalence of HBV markers was detected among the younger blood donors. Self-employed blood donors showed higher positivity for HBV markers compared to other line of works. Blood transfusion, sexual contact during window period or late phase chronic HBV infection are significant predictors of HBV infection among the study groups The presence of Hepatitis B virus in the blood of donors could pose some health risks on the blood recipients**.** There is need for inclusion of Hepatitis B profile test and the introduction of Polymerase chain reaction (PCR) in routine screening of blood donors in Nigeria in order to minimize the risk of transfusing blood with HBV infection.

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